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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants: Goelet et al.

Attorney Docket: 13017-3

Serial No.: 09/258,132

Examiner: Siew, J.

Dated Filed: February 26, 1999

Group Art Unit:1643

Title: "Nucleic Acid Typing by Polymerase Extension of Oligonucleotides Using Terminator Mixtures"

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Dated: December 3, 1999

Assistant Commissioner for Patents
Washington, DC 20231

PRELIMINARY AMENDMENT

Sir:

Prior to examination on the merits, please amend the application identified above as follows.

IN THE SPECIFICATION

At page 1, line 8, please insert; --This application is a continuation of application Serial Number 07/664,837, filed March 5, 1991, now U.S. Patent No. 5,888,819, and which is incorporated herein by reference.--

a1

IN THE CLAIMS

Please cancel claims 1-59.

Please add the following new claims 60-64.

a2

60. A method of determining the identity of one or more ~~nucleotide bases at specific positions of~~ one or more nucleic acid molecules of interest, comprising:

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(a) treating a sample comprising one or more nucleic acid molecules of interest, if the nucleic acid molecules of interest comprise double-stranded nucleic acid, so as to obtain unpaired nucleotide bases spanning the specific positions, or directly employing step (b) if the nucleic acid molecule of interest are single-stranded;

(b) contacting the sample from step (a) with one or more unique oligonucleotide primers, wherein;

A2 cont

(i) each unique oligonucleotide primer hybridizes, under high stringency hybridization conditions, to a different stretch of nucleotide bases present in the nucleic acid molecules of interest which is immediately adjacent to the nucleotide base to be identified with that unique oligonucleotide primer, so as to form a duplex such that the nucleotide base to be identified is the first unpaired base of the nucleic acid molecule of interest immediately downstream of the 3' end of the oligonucleotide primer, and

(ii) each unique oligonucleotide primer has a unique affinity moiety which permits affinity separation of the oligonucleotide primer from all the other oligonucleotide primers and wherein the affinity moiety specifically binds to a discrete position on a solid support, such discrete position is specific for the affinity moiety of the oligonucleotide primer;

(c) contacting the duplexes from step (b), in the absence of dATP, dCTP, dGTP, or dTTP, with four different terminators, each terminator comprising a different detectable label, of a nucleic acid template-dependent primer extension reaction, wherein one of the terminators is complementary to the nucleotide base to be identified by each of the oligonucleotide primers, wherein the contacting is under conditions sufficient to permit a template-dependent primer extension reaction which incorporates the complementary terminator onto the 3' end of each of the unique oligonucleotide primers to thereby extend the 3' end of each of the unique oligonucleotide primers by one terminator; and

(d) determining the presence and identity of the nucleotide base at the specific position or positions in each nucleic acid molecule of interest by detecting at each position of the solid support the detectable marker of the terminator incorporated at the 3' end of each of the unique oligonucleotide primers, such that each nucleotide base to be identified can be individually

identified by detecting at each position of the solid ~~support the~~ detectable marker of the terminator incorporated at the 3' end of each unique oligonucleotide primer.

61. A method according to claim 60, wherein the affinity moiety of each unique oligonucleotide primer comprises a sequence of nucleotides which permits attachment to the discrete positions on the solid support via base pairing of the sequence of nucleotides to its complementary sequence of nucleotides which are attached to the solid support at the discrete positions.

A2 cont

62. A method of determining the identity of one or more nucleotide bases at specific positions of one or more different nucleic acid molecules of interest, comprising:

(a) attaching a plurality of unique oligonucleotide primers to discrete positions of a solid support, wherein each unique oligonucleotide primer hybridizes, under high stringency hybridization conditions, to a different stretch of nucleotide bases present in the nucleic acid molecules of interest which is immediately adjacent to the nucleotide base to be identified with that unique oligonucleotide primer, so as to form a duplex such that the nucleotide base to be identified is the first unpaired base of the nucleic acid molecule of interest immediately downstream of the 3' end of the oligonucleotide primer;

(b) contacting the plurality of unique oligonucleotide primers of step (a) with a sample comprising a detectable amount of single stranded nucleic acid molecules of interest to form duplexes;

(c) contacting the duplexes from step (b), in the absence of dATP, dCTP, dGTP, or dTTP, with four different terminators, each terminator comprising a different detectable label, of a nucleic acid template-dependent primer extension reaction, wherein one of the terminators is complementary to the nucleotide base to be identified by each of the oligonucleotide primers, wherein the contacting is under conditions sufficient to permit a template-dependent primer extension reaction which incorporates the complementary terminator onto the 3' end of each of the unique oligonucleotide primers to thereby extend the 3' end of each of the unique

oligonucleotide primers by one terminator; and

(d) determining the presence and identity of the nucleotide base at the specific position in each nucleic acid molecule of interest by detecting the detectable marker of the terminator incorporated at the 3' end of each of the unique oligonucleotide primers which have been extended.

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64. A method of analyzing the sequence of nucleic acid molecules of interest, comprising:

(a) attaching a plurality of affinity moieties, wherein the affinity moiety comprises a unique sequence of nucleotides, to a solid support at defined positions;

(b) hybridizing the nucleic acid molecules of interest in solution to a plurality of oligonucleotide primers which comprise sequences of nucleotides complementary to the affinity moiety of step (a), under hybridization conditions, to generate a duplex;

(c) subjecting the hybridized primers to a template mediated single base primer extension reaction which comprises providing to the hybridized primers four terminators corresponding to each of the four nucleotide bases, to extend the hybridized primers by the addition of a terminator;

(d) sorting the extended primers by affinity capture by the affinity moieties of step (a);

(e) observing the identity and location of the terminator and thus determining the base at each of a plurality of sites of interest for the nucleic acid molecule of interest.

REMARKS

The new claims are directed to embodiments of the invention associated with identifying one or more nucleotide bases at specific positions of one or more nucleic acid molecules of interest. Support for these embodiments of the invention can be found throughout the specification. For example, affinity moieties on the primers are described at page 27, lines 12-24. The use of complementary nucleic acid sequences as affinity moieties for separating oligonucleotide primers on a solid support are specifically described, for example, at page 27,

lines 19-24. The use of multiple affinity moieties for separating one or more oligonucleotide primers is described, for example, on pages 29 and 30 of the specification. The use of affinity moieties at the 5' end of oligonucleotides to enable different affinity separations and thereby permit the analysis of several nucleic acid molecules of interest (oligonucleotides) at one time is described in the specification, for example, at page 31, lines 23-35. No new matter has been added.

It is respectfully submitted that this application is now in condition for further consideration and examination on the merits. If resolution of any remaining issue is required prior to examination of the application, it is respectfully requested that the Examiner contact Applicants' undersigned attorney at the telephone number provided below.

Respectfully submitted,

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